1. INTRODUCTION

" ... Je désire attirer votre attention sur les progrès réalisés au cours de ces dernières années par les études concernant les spirilles pathogènes. Il reste néanmoins de nombreux problèmes qui attendent encore leur solution, en particulier ceux qui ont trait à la nature intime de ces spirilles et surtout à la culture de ces micro-organismes."

LEVADITI (1908)

This work, a review of some of the available literature concerning the morphology, survival and culture of *Treponema pallidum* and associated organisms, appears within some 60 years of the final definition of *T. pallidum* as the cause of syphilis by Schaudinn & Hoffmann in 1905, and only shortly after the death of Erich Hoffmann in May 1959 at the age of 92 years. The above remarks of Levaditi, made at the XIVth International Congress of Hygiene held in Berlin in 1907, at which Schaudinn and Hoffmann were being widely congratulated on their discovery of only two years before, are just as applicable today, more than half a century later. The intimate nature of the organism, and the key to its successful cultivation outside the bodies of men or animals with the retention of its essential characteristics (i.e., virulence), still remain to be determined.

Much of what is known of the morphology of *T. pallidum* was described soon after Schaudinn & Hoffmann's discovery. Within a few years most of the aberrant forms sometimes ascribed to its development had been recorded (see Coutts & Coutts, 1953), and theories as to its life-cycle—controversial even today—were, as now, being widely propagated. Newer techniques such as the use of the electron microscope, instead of immediately simplifying matters, introduced at least for a time new controversies (e.g., on flagella).

T. pallidum was discovered relatively late for such a widespread and serious disease as syphilis, caused by a visible pathogen, and by the time of its discovery cultural techniques were already well established for other organisms. Only a year then passed before its successful cultivation was reported in 1906 (Volpino & Fontana, 1906; Schereschewsky, 1909), but these claims were soon found to be premature as far as the retention of virulence was concerned. Optimism about forthcoming success still prevailed, but during the following decades this gradually declined. Other treponemes from both mouth and genitals were freely cultivated *in vitro*—and indeed organisms morphologically resembling *T. pallidum* obtained from syphilitic lesions were successfully grown *in vitro* and in egg and tissue culture. Virulence, however, remained lacking, and the cultured organisms today resemble, or have come to resemble, saprophytic treponemes found in man.

It appears that, in those cases in which successful culture of virulent organisms was reported, this was due (on many occasions at least) merely to survival of the original organism in the medium and not to its multiplication. Noguchi (1911a) appeared to succeed in achieving a virulent culture of *T. pallidum*, but he was unable to reproduce his own results, and many others since have likewise failed. Many observers (e.g., Kast & Kolmer, 1943) consider that most of the so-called cultivations were not of *T. pallidum* but of morphologically and immunologically similar saprophytic treponemes. According to Eagle (1948) the cultured strains either never were *T. pallidum* or, if they were, have so changed that they are now in effect different organisms.

Which of these possibilities is correct is still uncertain, although it is of fundamental importance. If the cultivated strains *were* originally *T. pallidum*, then their loss of virulence is comparable with the well-known loss of virulence of pathogenic *T. pallidum* in body fluids and tissues removed from the host. If this proves to be the case, studies of the survival of virulent *T. pallidum*, and particularly of its virulence and of methods for prolonging it, are all-important as a possible key to its successful culture *in vitro*. Studies on the metabolism and growth requirements of the cultured forms could provide the vital clue. Also, morphological and immunological studies of the cultured strains might reveal differences which could be attributed to definable environmental influences. If the cultured strains are *not* derived from *T. pallidum*, although the study of their morphological, biological and immunological characteristics may show important points of difference, knowledge of the survival

factors of known virulent *T. pallidum* assumes even greater importance.

If it could be shown that the cultured strains do evolve from virulent *T. pallidum* in a demonstrable number of generations, this has application to the understanding of the modifications which may occur in *T. pallidum* as a result of environmental influences, and would support the concept of an evolutionary cycle in the development of the various disease syndromes of the treponematoses—yaws, endemic syphilis and pinta (Willcox, 1960)—and thus bring the time of divergence of the various responsible pathogenic strains from their ancestral treponeme to periods of relatively modern history rather than the dim and distant past (Cockburn, 1961). Certainly the apparent mutation of yaws treponemes to treponemes giving lesions of a more syphilitic type has been observed after numerous passages in rabbits by Manteufel & Herzberg (1929) with one strain, and by Turner & Hollander (1957) with two strains. With the achievement of successful culture it might be possible to accelerate this process *in vitro*.

Certainly, as is subsequently shown, much research has been undertaken in the attempted culture of *T. pallidum* and on studies of its survival and morphology through the years. The growth requirements of the cultivable strains have been determined, in many cases precisely; many of the growth requirements have been shown to be essential to the survival of pathogenic *T. pallidum*. The essential "break-through", however—that of successful cultivation of virulent *T. pallidum* outside the body—has not been achieved. Although the survival of virulent *T. pallidum* has been prolonged in artificial media, it still cannot be made to exceed what can occur in natural body fluids and tissues kept under similar conditions. Its virulence can be preserved in suspended animation for years at −78°C but this does not affect the argument.

POSSIBLE RESULTS OF SUCCESSFUL CULTURE OF VIRULENT *T. PALLIDUM*

The fruits of success in the culture of virulent *T. pallidum* outside the body would be manifold.

Successful culture would help to settle many of the controversial points of morphology, especially regarding the life-cycle of the organism, which could then be observed in the test-tube under varying environmental circumstances; it would help to resolve the problem of the existence or otherwise of an ultramicroscopic phase.

It would permit classification and clarification of the various disease syndromes—syphilis, yaws and pinta. Attempts could be made to change the character (mutation) of *T. pallidum* by varying the environmental conditions *in vitro*.

It would permit attempts at more accurate identification of treponemal forms recently found in lymph-node material of syphilitic man and animals following treatment (Collart et al., 1964).

It would permit the development of really specific but simple complement-fixation, flocculation and other serological tests based on a pure specific antigen, such as are at present performed with the Reiter treponeme. Tests in current use involving virulent treponemes from rabbits' testicles are cumbersome and expensive, and the results obtained may be confused by the presence of other substances in the antigen—including passive transfer of antibody (Turner & Hollander, 1957).

It would facilitate studies on the antigenic structure of the organism and of its enzyme systems.

It would allow the organ distribution of treponemes, especially in human late and latent syphilis, yaws and pinta, to be studied. *T. pallidum* has been demonstrated *post mortem* in the brain of man with general paralysis of the insane (Noguchi, 1913; Noguchi & Moore, 1913) and by means of animal inoculation of the blood of such patients (Levaditi, 1913). Attempts have also been made to isolate it from the brain *in vivo* (e.g., by Bessemans et al., 1951) and it has been successfully transferred to rabbits from miliary gummata in the aortic wall (Hu et al., 1946). Such results are often difficult to achieve, and the possession of an effective method of culture would enormously revive direct *in vivo* studies of organ localization.

It should permit the tagging of *T. pallidum* with radioactive isotopes (e.g., ^{32}P). Not only would this offer another means whereby organ distribution of treponemes in the body could be effectively examined (Rosahn, 1948), but also studies could be made of distribution of the organism in relation to that of therapeutic agents. To date, little work has been possible with radioactive isotopes, apart from attempts to reduce the serological reaction titre of rabbits (Mizumoto & Hayashi, 1956) and of humans (Nakajima & Hayashi, 1956) using ^{32}P, and limited studies on the tagging of treatment agents (e.g., bismuth by Musumeci et al., 1958).

Studies of sensitivity of the treponeme to various therapeutic substances, and on the possible development of penicillin resistance, could be made on a far more effective scale than is possible with existing methods.

Studies of the constituent chemistry of the treponeme would be facilitated by the possibility of providing large amounts of treponemal mass for analysis. Such studies would be combined with studies of antigenic structure.

Studies aimed at procuring active immunity against the treponemal diseases by vaccination procedures would be vastly widened in scope. Heretofore little has been achieved in this field. A number of workers have attempted to induce immunity in animals by the injection of killed organisms against syphilis in rabbits (Eagle & Fleischman, 1948; Magnuson et al., 1947; Waring & Fleming, 1951; Tani et al., 1951) and against yaws in monkeys (Schöbl, 1930; Schöbl et al., 1930). Gelperin (1951) used the Reiter treponeme in rabbits, and Wheeler (1960) tried various preparations obtained from pathogenic and non-pathogenic treponemes. Although no convincing evidence has been obtained of the production of artificial immunity, according to Turner & Hollander (1957) the work of Tani et al. (1951), Waring & Fleming (1951), Schöbl (1930) and Schöbl et al. (1930) has been " suggestive ". If no real immunity is induced by such procedures, demonstrable antibodies can, however, be invoked in rabbits (Eagle & Fleischman, 1948) and in mice (McLeod & Magnuson, 1951, 1953).

Noguchi (1911b, 1912) isolated a so-called pallida substance (luetin) from his cultured treponemes which was used in skin testing. Reiter (see *Lancet*, 1926) produced a vaccine from his cultures which also gave skin reactions. He tried it therapeutically in cases of general paralysis of the insane unimproved by other methods. Some improvement in the general state and in the serological status was claimed. However, such work, based on cultured or killed organisms, and unsuccessful attempts to produce immunity against syphilis in rabbits by inoculation with killed virulent treponemes, has not to date advanced very far (see Kolmer, 1930; McLeod & Magnuson, 1953; Magnuson et al., 1947).

Indeed, the conclusion is reached that there is hardly any problem of the vast subject of treponematosis research for which an advance would not be made towards solution by the successful *in vitro* culture of virulent *T. pallidum*.

WHO AND RESEARCH

Since its inception, the work of WHO has been closely bound up with research: that it was of basic importance to foster fundamental research for the long-term solution of field problems has been axiomatic. As the years have passed the importance of research has been even further emphasized and, at the Eleventh World Health Assembly in 1958, plans for intensified research were proposed in all sectors. The Director-General of WHO was then requested to prepare such plans for presentation to the Executive Board of WHO and to the Twelfth World Health Assembly.[1]

In the field of treponematoses the plans for an intensified research programme were welcomed by members of the WHO Expert Committee on Venereal Infections and Treponematoses at a meeting in September 1959 (World Health Organization, Expert Committee on Venereal Infections and Treponematoses, 1960), when some of the outstanding problems of research in the treponematosis field were considered, after preliminary consultation with leading workers in venereal diseases and treponematoses throughout the world.

The Committee indicated that continued research on the culture of pathogenic treponemes and the study of their antigenic structure were of paramount importance, as was work on the serological identification of active components in the treponemal body and on capsular material for differentiation between different groups and types of the micro-organism. Studies on survival of treponemes away from the body under different environmental conditions should also be given a high priority in the research programme.

The suggestions made by the Committee as to the most profitable lines of research, and others made in response to a request by WHO to members of its panels of experts and to other leading workers in this field, were considered by a specially formed WHO Scientific Group on Treponematoses Research [2] which was convened in November-December

[1] *Off. Rec. Wld Hlth Org.* **87**, WHA11.35.

[2] Members of the group were: Professor G. D'Alessandro, Director of the Institute of Hygiene and Microbiology, University of Palermo, Italy; Dr S. Hard, Karolinska Sjukhuset, Stockholm, Sweden; Dr P. H. Hardy, Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; Professor J. F. Murray, South African Institute for Medical Research, Johannesburg, South Africa (*Chairman*); Dr H. Aa. Nielsen, Director, Serological Department, Statens Seruminstitut, Copenhagen, Denmark; Dr W. G. Simpson, Assistant

(*continued overleaf*)

1959, to define areas of ignorance, and to consider priorities for research and make recommendations thereon.

The Group was conscious of a waning interest in the treponematoses owing to the fallacious belief that the introduction of penicillin had solved, or had rendered of lesser importance, the outstanding problems. Research had also gradually diminished and was now limited to very few countries and institutions. The Group believed that much too little was known of the true nature, extent and relationship of the different treponematoses, that important questions of morphology remained unanswered, and that the problem of cultivation of pathogenic treponemes *in vitro* was of the greatest importance from many points of view. The *in vitro* growth and characterization of pathogenic treponemes would, it was considered, assist in their classification, especially of those responsible for the disease syndromes yaws, syphilis and pinta. With present immunological methods it is barely possible to distinguish between the organisms of those species which can be propagated in the experimental animal.

The Group affirmed that the successful cultivation of *T. pallidum* would allow critical laboratory studies on penicillin sensitivity and on possible mutation of treponemes, such as induced penicillin-resistance, to be carried out, and it would facilitate studies of antigenic structures and enzyme systems which at present are difficult to undertake because of the very limited treponemal mass available, from the enforced use of infective animal material. The Group concluded that although the problem of cultivation was undoubtedly complex, it was of basic importance for the advancement of fundamental knowledge of the treponematoses, and that co-ordinated efforts between interested laboratories should be made in an intensified research programme, designed to cultivate the treponeme *in vitro*.

Part of the recommendations of the WHO Scientific Group on Treponematoses Research concerned the necessary reference and supporting services of a research programme. A WHO Advisory Committee on Medical Research, which later reviewed the plans, gave high priority to this aspect. The Scientific Group stressed the importance of a free exchange of information between interested workers, and considered that WHO should continue to assist by furnishing technical bulletins of the type at present issued by the Organization. Bibliographies on limited subjects of interest to research workers in the treponematoses field should be also made available. As a first step it was suggested that a thorough review of the literature in regard to previous cultivation attempts should be made.

As a preliminary move members of the Scientific Group and other persons engaged in fundamental treponematoses research were asked to supply references concerned with the morphology, survival and cultivation of *T. pallidum* and associated treponemes to serve as a basis on which this fuller bibliography could be compiled. WHO acknowledges with appreciation the help given by those concerned. On these foundations this review has been assembled.

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2. TAXONOMY [1]

The name "spirochaete" was first given by Ehrenberg in 1838 to large, free-living, flexible organisms floating in fresh and marine water, e.g., *Spirochaeta plicatilis*. Spirally-shaped organisms were for a long time grouped under the common name of *Spirillum*, *Spirochaeta* or *Vibrio*. Later

[1] See Wilson & Miles (1955), Wenyon (1926), Jordan & Burrows (1945).